REVIEW

Endothelins as local activators of adrenocortical cells

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Abstract

Besides the classical corticotrophic hormones, ACTH and angiotensin II, various regulatory peptides produced by the adrenal gland are thought to participate in the control of corticosteroid secretion. Here, we review the evidence that endothelins (ETs) synthesized within the adrenal cortex may act as autocrine and/or paracrine factors to regulate adrenocortical cell activity. The expression of ETs has been detected in normal, hyperplastic and neoplastic adrenocortical cells. The occurrence of ET receptors has been described in the different zones of the cortex. ETs stimulate the secretion of both glucocorticoids and mineralocorticoids, and modulate the proliferation of adrenocortical cells. The effects of ETs on steroidogenic cells are mediated through the activation of various signaling mechanisms including stimulation of phospholipase C, phospholipase A2 and adenylyl cyclase activity, as well as calcium influx through plasma channels. These observations suggest that locally produced ETs may play an important role in the regulation of corticosteroid secretion and in the control of mitogenesis in normal and tumoral adrenocortical cells.

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Introduction

The endothelin (ET) family is composed of three related peptides, ET-1, ET-2 and ET-3, each of which comprises 21 amino acids with two intramolecular disulfide bridges. The sequences of ET-2 and ET-3 only differ from that of ET-1 by two and six amino acids respectively (Yanagisawa et al. 1988). All three biological isoforms are generated via a two-step processing pathway: (1) prepro-ETs are cleaved at dibasic residue sites by prohormone- and furin-like convertases to form the physiologically inactive ET precursors designated pro-ETs or big-ETs and (2) specific endopeptidases, termed endothelin-converting enzymes (ECEs), hydrolyze the Trp21-Val22 bond (Trp21-Ile22 bond in big ET-3) to generate the mature forms of ETs (Fig. 1). The effects of ETs are mediated through interaction with at least three types of G-protein-coupled membrane receptors. Two receptor subtypes, called ET_A and ET_B, have been cloned in mammals (Arai et al. 1990, Sakurai et al. 1990). The ET_A receptor exhibits a higher affinity for ET-1 and ET-2 than for ET-3, whereas the ET_B receptor does not discriminate between the three isoforms. A third receptor, called ET_C, that binds ET-3 with high affinity, has been identified on Xenopus laevis dermal melanophores (Karne et al. 1993), but the biological significance of this receptor is still unclear. Besides their well-established vascular effects, ETs display a large array of biological actions. ETs have been reported to modulate the activities of various endocrine glands including the anterior pituitary, thyroid, parathyroid, gonads and
adrenal gland (Masaki 1993). The purpose of the present review is to summarize the evidence concerning the involvement of ETs in the regulation of corticosteroid secretion.

**Presence of ETs in the adrenal gland**

The occurrence of ETs in adrenal tissue has been reported for several mammalian species (Nunez et al. 1990, Sakurai et al. 1991, Imai et al. 1992). Specifically, ET-1, ET-3 and their respective ECEs (ECE-1 and ECE-3) have been detected in adrenal homogenates (Imai et al. 1992, Rossi et al. 1995) and in dispersed human adrenocortical cells (Rossi et al. 1997a). It has also been reported that ECE-1 is expressed in the human adrenal cortex and medulla, the highest concentration being detected in the zona glomerulosa (ZG) (Korth et al. 1999). The presence of immunoreactive ET-1 in human adrenocortical cells, notably in the zona fasciculata (ZF), but not in the medulla (Li et al. 1994, 1995,

![Figure 1: Schematic representation of the organization of the human ET-1 gene, mRNA, precursor protein, and processing pathway. The human ET-1 prepropeptide encompasses 212 amino acids. Biosynthesis of ET-1 involves three successive steps: (1) prohormone convertases cleave the precursor at the Arg<sup>52</sup>-Cys<sup>53</sup> and Arg<sup>92</sup>-Ala<sup>93</sup> bonds, (2) a carboxypeptidase removes the Arg<sup>92</sup> and Lys<sup>91</sup> residues from the COOH terminus to yield pro-ET-1 or big ET-1, and (3) endothelin-converting enzyme 1 (ECE-1) catalyzes the conversion of big ET-1 to ET-1 by cleaving the Trp<sup>73</sup>-Val<sup>74</sup> bond.](image-url)
Hiraki et al. 1997), has been demonstrated. The occurrence of ET-1 in the human adrenal gland has been confirmed by HPLC analysis while ET-3 has not been detected (Davenport et al. 1996). The expression of prepro-ET-1 and prepro-ET-3 mRNA has been reported in the adrenal gland from patients with aldosterone-producing adenoma (Imai et al. 1992). Although the ET genes are not expressed in the normal adrenal medulla, both ET-1 and ET-3 have been detected in pheochromocytoma tissue (Watanabe et al. 1997) and it has been proposed that ETs may contribute to the hypersecretion of catecholamines in pheochromocytomas (Zeng et al. 1998). Expression of prepro-ET-1 and ECE-1 in human adrenocortical carcinoma NCI-H295 cells has also been shown (Rossi et al. 1997b). The recent observation that human adrenocortical carcinoma SW-13 cells produce and secrete ET-1 (Takahashi et al. 2000) provides additional evidence for a possible physio-pathological implication of ET-1 in the regulation of corticosteroid secretion. ETs have been detected in the adrenal gland of the European green frog, Rana ridibunda (Wang et al. 2000). Interestingly, amino acid sequence analysis has shown that frog ET-1 is identical in structure to human ET-1, while frog ET-3 only differs from human ET-3 by a single substitution (Wang et al. 2000), indicating that the primary structure of ETs has been remarkably well conserved during the evolution of the tetrapods.

Effects of ETs on the adrenal cortex

There is now clear evidence that ETs are potent stimulators of corticosteroid secretion in various mammalian species. All three ET isoforms enhance aldosterone production by the rat adrenal gland (Hinson et al. 1991a). ET-1 has been reported to stimulate aldosterone output in rabbit (Morishita et al. 1989) and human (Zeng et al. 1992) adrenocortical cells, as well as in Conn’s adenoma (Rossi et al. 2000). In contrast, ET-1 has no effect on aldosterone secretion from cultured bovine glomerulosa cells (Rosolowsky & Campbell 1990). Studies aimed at investigating the possible involvement of ETs in the regulation of glucocorticoid secretion by the human adrenal gland have led to controversial results. ET-1 and ET-3 were found to stimulate cortisol secretion from isolated human adrenocortical cells (Hinson et al. 1991b) whereas ET-1 had apparently no effect on cortisol output from adrenal slices (Zeng et al. 1992). In addition to its direct effect on corticosteroid secretion, a possible indirect effect of ET-1 through activation of chromaffin cells in rat, bovine and canine adrenal glands has also been documented (Nussdorfer et al. 1999). Infusion of ET-1 to healthy volunteers does not affect basal plasma cortisol and aldosterone levels, but potentiates the stimulatory effect of adrenocorticotropicin (ACTH) on aldosterone concentrations (Vierhapper et al. 1995). Besides its action on the secretory activity of the adrenal gland, ET-1 causes proliferation of rat ZG cells (Malendowicz et al. 1997) and this mitogenic action is mimicked in vitro by the C-terminally elongated form of ET-1, ET-1[1–31] (Rebuffat et al. 2001a). Conversely, ET-1 exerts a marked antimitogenic effect on cultured bovine adrenocortical cells (Cozza & Gomez-Sanchez 1990).

In contrast to mammals, the frog adrenal gland does not exhibit any zonation but is composed of intermingled steroidogenic and chromaffin cells (Leboulenger et al. 1983). This particular anatomical organization favors interactions between adrenochromaffin and adrenocortical cells. Early studies have shown that in the frog, Rana ridibunda, ET-1 is a potent stimulator of corticosterone and aldosterone secretion from perfused adrenal slices (Delarue et al. 1990). The integrity of the microfilament network is required for the corticotropic activity of ET-1 whereas microtubules and intermediate filaments are apparently not involved (Remy-Jouet et al. 1994). ET-3 also stimulates corticosteroid secretion by frog adrenal tissue but the potency of ET-3 is lower than that of ET-1 (Wang et al. 2000). Taken together, these data indicate that the corticotropic effect of ETs has appeared early during evolution and that ETs may play a significant role in the local regulation of corticosteroid secretion in normal and pathological conditions.

Characterization of endothelin receptor subtypes in the adrenal gland

Autoradiographic studies performed in pig, rat and human adrenal gland have shown that the
concentration of ET-1 binding sites is about twofold higher in the ZG than in ZF/zona reticularis (ZR) (Davenport et al. 1989). Subsequently, it has been demonstrated that the rat and human ZG, as well as the medulla, contain both ET\textsubscript{A} and ET\textsubscript{B} receptors whereas the ZF/ZR possesses only ET\textsubscript{B} receptors (Rossi et al. 1994, Belloni et al. 1997). The presence of ET\textsubscript{A} and ET\textsubscript{B} receptors has also been observed in aldosterone-producing adenoma and pheochromocytoma (Rossi et al. 1994, Watanabe et al. 1997). However, the type of receptor mediating the corticotropic effects of ETs is variable. For instance, ET-1 enhances aldosterone secretion from bovine adrenocortical cells through activation of ET\textsubscript{A} receptors (Naruse et al. 1994). In contrast, it has been reported that, in the rat, the stimulatory effect of ET-1 on mineralocorticoid secretion can be accounted for by activation of either the ET\textsubscript{B} receptor only (Belloni et al. 1996, 1997, Pecci et al. 1998) or by both the ET\textsubscript{A} and ET\textsubscript{B} receptors (Kapas et al. 1996), whereas ET\textsubscript{A} receptors mediate exclusively the stimulatory effect of ET-1 on glomerulosa cell proliferation (Mazzocchi et al. 1997). In human adrenocortical cells, ET\textsubscript{A} and ET\textsubscript{B} receptors seem to contribute equally to the activation of ET receptors (Rossi et al. 1997\textsubscript{a}, Rebuffat et al. 2000\textsubscript{b}). Similarly, in Conn’s adenoma, the effect of ET-1 on aldosterone secretion likely involves both receptor subtypes (Rossi et al. 2000). Finally, in frog, the effects of ET-1 and ET-3 on corticosteroid secretion are mediated by an ET\textsubscript{A}-like receptor (Cartier et al. 1997). These findings indicate the existence of species differences in the type of receptor involved in the corticotropic effect of ETs in vertebrates.

**Signaling pathways associated with the activation of ET receptors**

The action of ETs on ET\textsubscript{A} and ET\textsubscript{B} receptors is generally mediated through phospholipase C (PLC) activation. In amphibians as in mammals, the stimulatory effect of ET-1 on corticosteroid secretion is associated with an increase in PLC/protein kinase C (PKC) (Pouzeratte et al. 1998, Cartier et al. 1999, Andreis et al. 2001) (Fig. 2). In rat glomerulosa cells, ET-1-stimulated aldosterone production is mediated through PKC activation of protein tyrosine kinase (PTK) activity (Kapas & Hinson 1996). It has recently been shown that, in human adrenocortical cells (Rebuffat et al. 2001\textsubscript{b}, Andreis et al. 2002), as in Conn’s adenoma (Rossi et al. 2000), ET-1-evoked corticosteroid secretion is mediated through activation of ET\textsubscript{A} receptors coupled to PLC and of ET\textsubscript{B} receptors coupled to both the PLC/PKC and the cyclooxygenase cascades. However, nothing is currently known regarding the type of G-protein that couples the ET receptors to the enzymatic signaling pathways. While ET-1 has no effect on cytosolic calcium concentration ([Ca\textsuperscript{2+}]\textsubscript{i}) in cultured bovine ZG cells (Rosolowsky & Campbell 1990), Ca\textsuperscript{2+} is clearly involved in the action of ET-1 on adrenocortical cells in other species. ET-1 has been shown to raise [Ca\textsuperscript{2+}]\textsubscript{i}, in dispersed rat (Andreis et al. 2001) and cultured human (Pouzeratte et al. 1998) ZG cells. The L-type Ca\textsuperscript{2+} channel antagonist, nicardipine, was found to inhibit ET-1-evoked aldosterone secretion by dispersed rabbit adrenal cells (Morishita et al. 1989) and human adrenal slices (Zeng et al. 1992). The L-type calcium channel blocker, verapamil, also reduced the corticotropic effect of ET-1 in cultured calf ZG cells (Cozza & Gomez-Sanchez 1993). In frog adrenocortical cells, the stimulatory effect of ET-1 on steroid secretion is mediated by an ET\textsubscript{A} receptor coupled to adenylyl cyclase and PLC (Cartier et al. 1999). Activation of PLC induces calcium mobilization from intracellular stores while stimulation of the adenylyl cyclase/protein kinase A (PKA) cascade causes phosphorylation of L-type calcium channels leading to calcium influx through the plasma membrane of frog adrenocortical cells (authors’ unpublished data). Concurrently, in the frog adrenal gland, ET-1 activates the cyclooxygenase pathway (Delarue et al. 1990). Thus, it appears that while the stimulatory effect of ETs on corticosteroid secretion is a common feature throughout vertebrates, the transduction mechanisms associated with receptor activation exhibit marked species differences (Fig. 2).

The proliferative effects of ETs on rat adrenocortical cells are mediated through PKC- and PTK-dependent signaling pathways (Mazzocchi et al. 1997). In particular, it has been reported that the intermediate processing product ET-1[1–31] exerts its mitogenic effects on zona glomerulosa cells through stimulation of PTK- and PKC-dependent activation of the p42/p44 MAP-kinase cascade (Mazzocchi et al. 2000).

Conclusion

Increasing evidence suggests that, in addition to their well-known cardiovascular effects, ETs act as autocrine or/and paracrine regulators of corticosteroid secretion. ETs have been reported (1) to be synthesized in the adrenal gland, (2) to stimulate glucocorticoid and/or mineralocorticoid secretion through activation of ET<sub>A</sub> and ET<sub>B</sub> receptors, (3) to activate several signaling pathways in adrenocortical cells, and (4) to exert mitogenic effects on ZG cells. The fact that ET-1 exerts a dual effect on the adrenal gland, i.e., a stimulatory effect on corticosteroid secretion and a mitogenic action on adrenocortical cells, suggests the possible involvement of ETs in the pathogenesis of idiopathic hyperaldosteronism as well as in the growth of adrenal tumors.

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